IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Frasch; Wayne D.; et al.	Confirmation No. 4137
Application No.: 10/538534	Art Unit: 1634
Filed: 6/10/2005	Examiner:
Title: Polarization-Enhanced Detector with Gold Nanorods for Detecting Nanoscale Rotational Motion and Method Therefor	SHAW, AMANDA MARIE
Attorney Docket No.: 60227US	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TRANSMITTAL LETTER OF STATEMENT UNDER 37 C.F.R. § 1.132

Dear Commissioner:

Attached is the Statement of Dr. Wolfgang Junge in support of the present application. Dr. Junge is a Professor of Biophysics at the University of Osnabrück, Germany. A copy of Dr. Junge's C.V./publication/citation list is attached to his Statement. This Statement was not presented earlier as we have just received it from Dr. Junge.

Dr. Junge is among the pioneers of time resolved-polarized photometry, single molecule detection and nanomechanics in biology, from 1972 to the present and has conducted research and development in what is commonly known as Biophysics, and, here in particular, structure and dynamics of enzymes and membranes, biological devices and materials that exist and operate in the range of 1 to 1000 nanometers (nm). Many of the methods developed and applied in this field have been later extended into modern nanotechnology. It has been said that the ultimate refinement of realization and sensitivity is a single molecule. With nanotechnology, work is done at the molecular level. Complex processes can take place in such a small space that the application become very portable. Propagation times and energy consumption are negligible.

As stated by Dr. Junge, one of the behavioral phenomena that exist in the world of nanotechnology is continuous stochastic motion overlaid to any directed motion. The nature of the motion is directly related to the physical characteristics and environment to which the nanoscale elements and structures are subjected. The ability to detect, observe, measure, and control such motion at the nanometer scale is important to the continuation of research and development of new products and design methods. The development and application of new instrumentation and research techniques for the accurate and reliable detection of motion, particularly rotational motion, in the nanometer range is essential for progress in nanotechnology.

Rotary F-ATPase, the essential 'turbo-generator' of the power stations of the cell, has become the test-field for the development of tools for the detection of nanoscale rotation. Dr. Junge has used polarized photobleaching and depolarization for high time resolution of rotational motion. This technique, applicable to a large ensemble of molecules did not reveal sub-steps of motion in a given single molecule, a limitation that was overcome by a Japanese team who made rotation in a single molecule visible in a fluorescence microscope. Originally they attached muscle fibers of 0.5 to 4 μm length to the rotating shaft of the enzyme, later anisotropically patterned fluorescent polymer microspheres in the range of 2 to 4 μm in diameter and video-graphed their rotation as driven by the central shaft of this enzyme. This approach has been extended to non-fluorescent but light scattering polystyrene spheres. A general drawback has been the diffraction limit of resolution by the light microscope (half the light wavelength in standard design). This limitation has been overcome by focusing the video-evaluation on the centroid of the diffuse diffraction image of the reporter bead/filament. The above techniques, later also used by other laboratories, were greatly successful in characterizing the dynamic and structural properties of this paradigmatic rotary enzyme.

The single-molecule fluorescence polarization spectroscopy and the centroid-tracking method share limitations in that the detected signal is weak because fluorescence probes are susceptible to photo-bleaching, and dielectric light scatterers

are ineffective. The weak signal-to-noise has been improved by using the more stable quantum dots (nano-scaled semiconductor particles) instead of organic fluorophores. In general, it is difficult to observe rotation of a circular object at any scale when viewed along the axis of rotation unless the rotation of the object is eccentric to the axis of rotation and/or the rotating object has an asymmetric shape. The detection of rotating molecules with appropriately attached and sufficiently bright probes has been a very time and manpower consuming task lacking the properties for easy and automated processing. The impressive insights on the dynamics of rotary F-ATPase that have been gained by the above techniques have required to handle two obstacles, first finding 'the blurred needle in the haystack' and then studying it over an often too short time interval.

The shortcomings detailed in the above two paragraphs, namely (i) limited signal-to-noise, (ii) the necessity to interpret blurred images, and (iii) often too short observation intervals were to a large part overcome by the group of Wayne Frasch. They used gold nanorods. Gold nanorods have the following favourable properties, they are (i) high-intensity light scatterers, (ii) intrinsically of elongated shape, and (iii) they scatter light in two different colors, green and red, depending on whether the short or the long axis of the rod is parallel to the electric vector of the exciting light. The rotation of the rod around an axis perpendicular to its long one is therefore apparent as an alternation of green and red scattered light. Because of small size there is little viscous drag on the rod, and it follows the rotary drive with little delay, thus reporting very fast steps of rotation (microsecond time resolution!). Although this new technique lacks the beauty of video-graphed motion pictures of rotary single molecule as introduced by Noji et al. in 1997 it is superior for automated detection, long-term observation and high time resolution, all of which are properties desirable for nanotechnological applications.

It is true that two elements of the present patent application were pioneered by others, the light scattering properties of nanorods were described by Sonnichsen, and the bead-to-F-ATPase construct by Noji, Yasuda et al. Still their combination has not been attempted by the various Japanese groups that have emerged from the

laboratories of Masasuke Yoshida and Katsuhiko Kinosita in a project series that has been pushed forward in many different directions, most generously funded by the Japanese authorities. The combination of these two elements is an original contribution by Wayne Frasch. It has led his groups to an unparalleled high resolution of hitherto unresolved substeps (by 36°) in the electromotor-portion of F-ATPase. Most importantly, the intensity of scattered light, alternating between red and green, was more brilliant and longer lasting than in any previous approach. Therewith automated detection and evaluation and nanotechnical applications beyond the F-ATPase are in close reach.

Over the last ten years up to now Dr. Junge has reviewed for top journals the manuscripts of the pertinent groups in Germany, Japan, the UK and the US, and I have met the respective principle investigators at conferences. Up to the time of signing his Statement he was not aware of a duplication of the Frasch-technique by any other group.

According to Dr. Junge, the superiority and novelty of the method of the present invention is evident from the following: (i) The pioneering and still leading big groups in Japan have so far not attempted to resolve the small sub-steps of the electromotor of F-ATPase. (ii) A competing group in Germany, Michael Börsch's, has obtained the first rather limited resolution of these steps by a fluorescence technique (FRET), being hardly convincing to the biochemical community, and (iii) the resolution in Wayne Frasch's method is by far superior. He has critically and constructively reviewed the latter two manuscripts for EMBO journal.

Since at least as early as 2000 there has been a need for a viable detection system to automatically identify and observe over long time intervals single phase molecules that has been unmet until the solution provided by the present invention.

He goes on to state that, although progress was being made concerning the development of nanoparticles (Mock and Sonnichsen) at the same time as that of single molecule rotation measurements by Noji, Yasuda, Yoshida, and Kinosita, and now also in the hands of Futai in Japan - Börsch, Gräber, Junge in Germany - Montemagno in

USA, the dissemination of knowledge between these groups was slow. Each group in this competitive field kept to itself, communication between them was limited, even when sharing a common HFSP grant (e.g between Junge & Yoshida). None of them considered the nanorod rotation. Although all these groups were involved in funding schemes for nanotechnical applications (e.g. of magnetically driven ATP synthesis, F-ATPase based nano-transport devices) none of them considered a high sensitivity application by merging the rotary motor technology (Noji, noted above), nanorod scattering technology (Sonnichsen, Physical Review Letter Pub 112002) and ultra-sensitive DNA detection (Felder, US Patent 6,232,066). The innovative aspect of this application is the successful merger, that has required extensive technical effort beyond that developed by its predecessors.

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Date

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